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AMENDMENTS TO THE SPECIFICATION

Please amend paragraphs [00182] to [00189] in the specification as follows:

Example 1. Expression and Purification of FAPα (SEQ ID NO:3)

[00182] This example describes cloning, expression and purification of ~~FAP□~~ FAPα (SEQ ID NO:3). It should be noted that a variety of other expression systems and hosts are also suitable for the expression of ~~FAP□~~ FAPα, as would be readily appreciated by one of skill in the art.

[00183] The portion of the gene encoding residues 27-760 (from SEQ ID NO:1), which corresponds to the extracellular portion of human ~~FAP□~~ FAPα, was amplified by PCR from IMAGE clone 4731931 (ATCC 6670182). The primers used in the PCR reaction incorporated an N-terminal SmaI restriction site and a C-terminal 6xHis tag, stop codon and SmaI restriction site. The PCR product was digested with SmaI and ligated into an appropriately digested modified pFastBacHTb vector. The final construct encodes a baculovirus glycoprotein gp67 signal peptide sequence at the N-terminus followed by the 27-760 ~~FAP□~~ FAPα sequence (SEQ ID NO:2), a 6x-histidine tag sequence and stop sequence at the C-terminus. Expression in this vector allowed for the production of secreted recombinant ~~FAP□~~ FAPα with part of the signal sequence at the N-terminus and a 6x-histidine tag at the C-terminus, the sequence of which is shown in Figure 1 (part of signal sequence and 6x-histidine tag sequence underlined) (SEQ ID NO:3). Recombinant baculovirus genomic DNAs incorporating the FAP cDNA sequences were generated by transposition using the Bac-to-Bac system (Gibco-BRL). Infectious extracellular virus particles were obtained by transfection of a 2 ml adherent culture of Spodoptera frugiperda Sf9 insect cells with the recombinant viral genomic DNA. Growth in ESF 921 protein free medium (Expression Systems) was for 3 days at 27°C. The resulting passage 1 viral supernatant was used to obtain passage 2 high titer viral stock (HTS) by infection of a 2 ml adherent culture of Spodoptera frugiperda Sf9 insect cells grown under similar conditions. Passage 2 HTS was used in turn to infect a 100 ml suspension culture of Spodoptera frugiperda Sf9 insect cells in order to generate passage 3 HTS. The production of recombinant FAP proteins was carried out using the passage 3 HTS at a multiplicity of infection (moi) of approximately 5 to infect 0.5-5 liter cultures of Trichoplusia ni Hi5 insect cells (InVitrogen) at a cell density of (1.5-8)x10^6 cells/ml (grown in ESF 921 protein free medium). Infected 5L cell cultures were grown in WAVE BIOREACTORS® (Wave Biotech) for 48 hours at 27°C prior to harvest.

[00184] Following harvest, the cell cultures were centrifuged to pellet whole cells and the culture medium supernatant was concentrated using a cross-flow ultrafiltration apparatus. In a typical batch preparation, 5 L of cell culture supernatant were concentrated to 0.1 L on a 10 kDa NMWCO Omega ULTRASETTE® (Pall Life Sciences) using a MASTERFLEX® L/S pump fitted with PharMed #15 tubing at a cross flow of approximately 1 L/min and an inlet feed pressure of 1.5 to 2.0 bar, generating an initial permeate flow of up to 70 ml/min. The retentate was diluted two to three-fold by adding 25 mM Tris/HCl pH 7.9, 0.4 M NaCl and reconcentrated to 0.1 L. This process was repeated at least twice, after which the concentrate was quantitatively removed from the system, centrifuged when necessary (15 minutes at 4,000 rpm in an ALLEGRA® (Beckman) centrifuge) and added to approximately 8 ml of a preconditioned 50 % slurry of PROBOND® (Invitrogen) divided over three or four 50ml conical tubes. The tubes were rotated for at least 1 hour, after which the resin was washed with 10 resin volumes of 50 mM Potassium Phosphate pH 7.9, 0.4 M NaCl, 0.25 mM TCEP. The resin was poured into 1 cm ID glass columns (Omnifit) and washed with 50 column volumes of 50 mM Potassium Phosphate pH 7.9, 0.4 M NaCl, 20 mM imidazole, 0.25 mM TCEP. After a wash with 5 column volumes of 50 mM Tris pH 7.9, 0.4 M NaCl, 0.25 mM TCEP, the product is eluted with 4 column volumes of 50 mM Tris pH 7.9, 0.4 M NaCl, 200 mM imidazole, 0.25 mM TCEP. It is noted that for the purification of non-secreted proteins, leupeptin is added to all the buffers used during the IMAC process at 1 mg/L and that for simplicity reasons the same is sometimes done when purifying ~~FAP□~~ FAPα (SEQ ID NO:3).

[00185] After concentrating ~~FAP□~~ FAPα (SEQ ID NO:3) by centrifugal ultrafiltration (10 kDa NMWCO, VivaScience), the protein was purified further by passage over a BIOSEP Sec S3000 column (200mm x 21.2 mm, Phenomenex) at 8 ml/min to remove high molecular mass material. The column was set up in a SUMMIT® HPLC system (Dionex) managed by CHROMELEON® software (Dionex) and equilibrated with 25 mM Tris pH 7.6, 150 to 250mM NaCl, 0.25 mM TCEP and 1 mM EDTA. ~~FAP□~~ FAPα (SEQ ID NO:3) was concentrated to a final concentration of 8 mg/mL or greater by centrifugal ultrafiltration (10 kDa NMWCO, VivaScience).

[00186] The process was carried out at 2–10°C and ~~FAP□~~ FAPα was stored at the same temperature. For long-term storage, ~~FAP□~~ FAPα (SEQ ID NO:3) was kept at –80°C. The ~~FAP□~~ FAPα (SEQ ID NO:3) purity as determined by SDS-PAGE was at least 95%.

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Example 2. Crystallization of FAPα (SEQ ID NO:3)

[00187] This example describes the crystallization of ~~FAP□~~ FAPα (SEQ ID NO:3). It is noted that the precise crystallization conditions used may be further varied, for example by performing a fine screen based on these crystallization conditions.

[00188] Crystals were obtained after an extensive and broad screen of conditions, followed by optimization. Diffraction quality crystals were grown in 100nL sitting droplets using the vapor diffusion method. 50nL comprising the apo ~~FAP□~~ FAPα (SEQ ID NO:3) (between 8 and 30 mg/ml) was mixed with 50nL from a reservoir solution (100μL) comprising: 0.05M CHES, pH=9.5; 22% PEG 6000; and 1.26M LiCl. The resulting solution was incubated over a period of two weeks at 4°C.

[00189] Crystals typically appeared after 3-5 days and grew to a maximum size within 7-10 days. Single crystals were transferred, briefly, into a cryoprotecting solution containing the reservoir solution supplemented with 25% v/v ethylene glycol. Crystals were then flash frozen by immersion in liquid nitrogen and then stored under liquid nitrogen. A crystal of apo ~~FAP□~~ FAPα (SEQ ID NO:3) produced as described is illustrated in Figure 2.